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GB 1283331 A EP 0040420 A1 WO 91/15200 A2  
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(54) **Therapeutic arylating agents**

(57) Arylating agents active against cancer and viral infections e.g. aids have an aromatic ring having at least one labile leaving group and at least one electrophilic group. Typical agents are benzenesulphonic acids, dinitrobenzenes, nitroanilines, nitrophenols, halogenated and nitro benzoic acids, chloronitro benzamides.

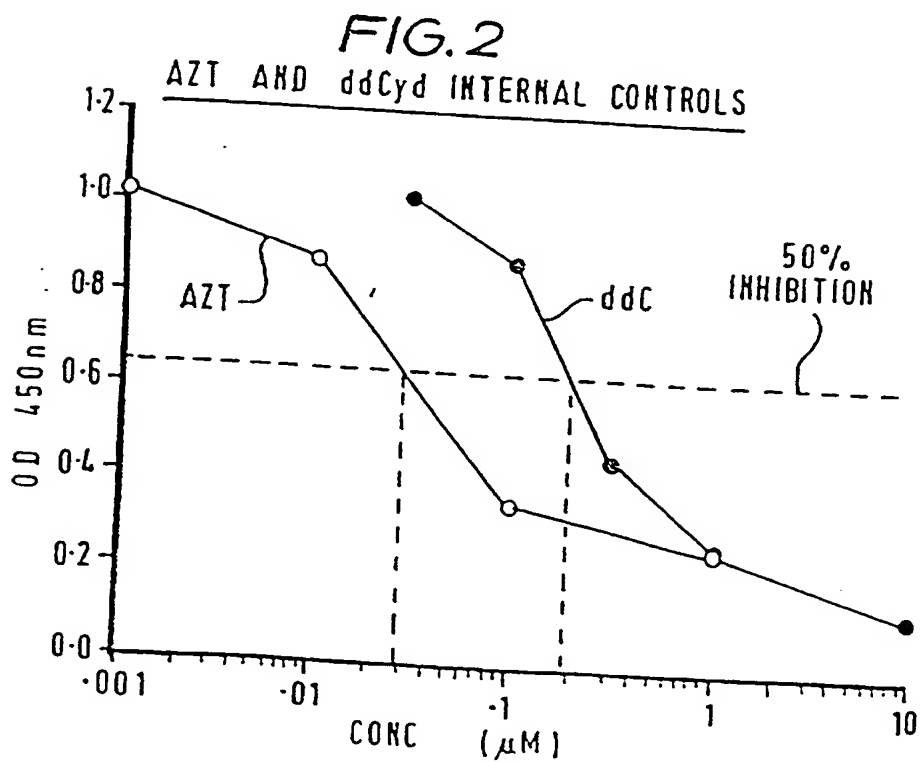
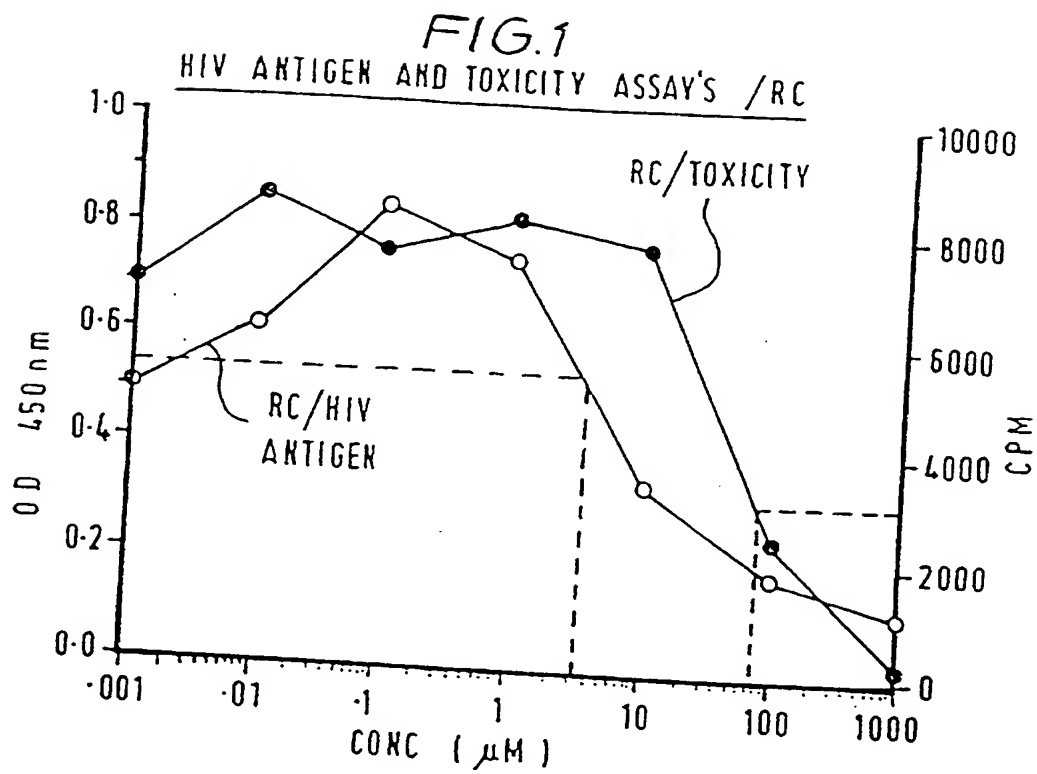


FIG. 3

THE EFFECTS OF 2,4-DICHLORO, 3,5-DINITROBENZOIC ACID ON  
 THE GROWTH OF MAC13 MURINE ADENOCARCINOMA COLON TRANSPLANTED  
 SC AND DRUG ADMINISTERED IP USING A SPLIT-DOSE SCHEDULE  
 6 ANIMALS PER GROUP

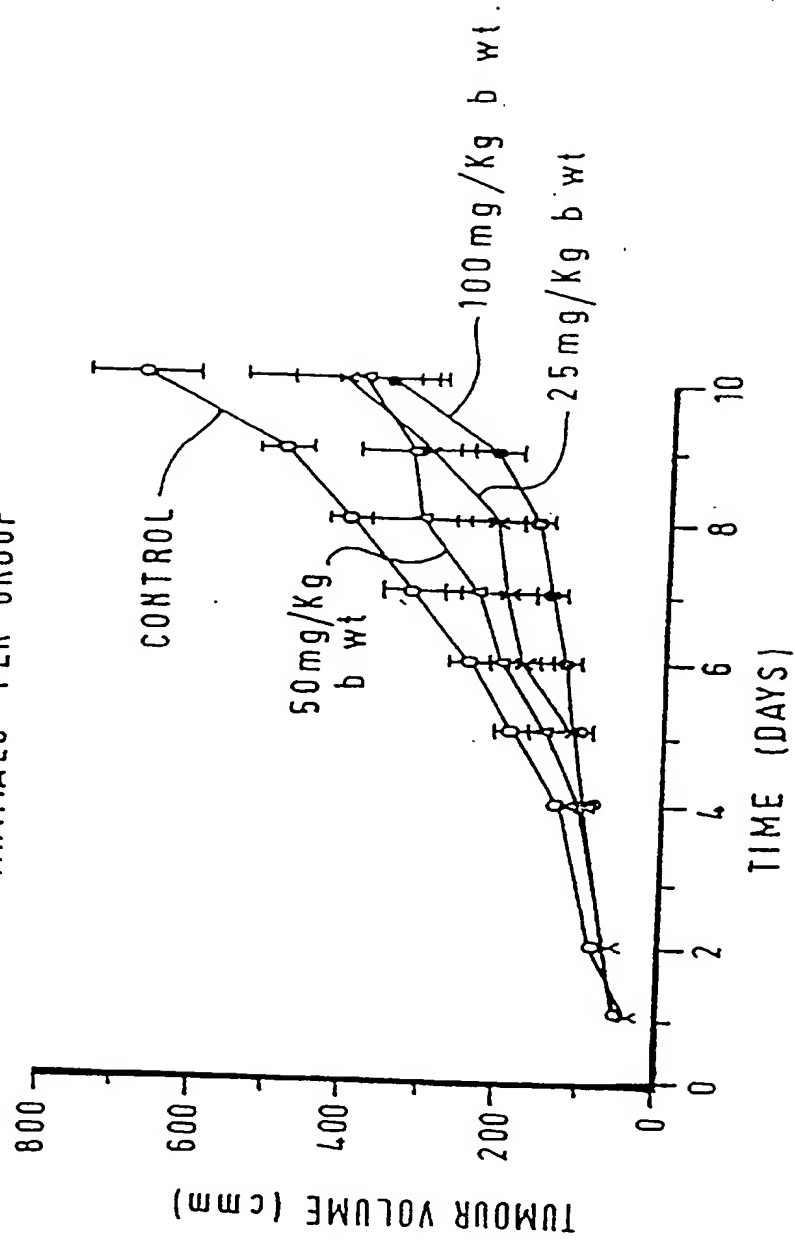
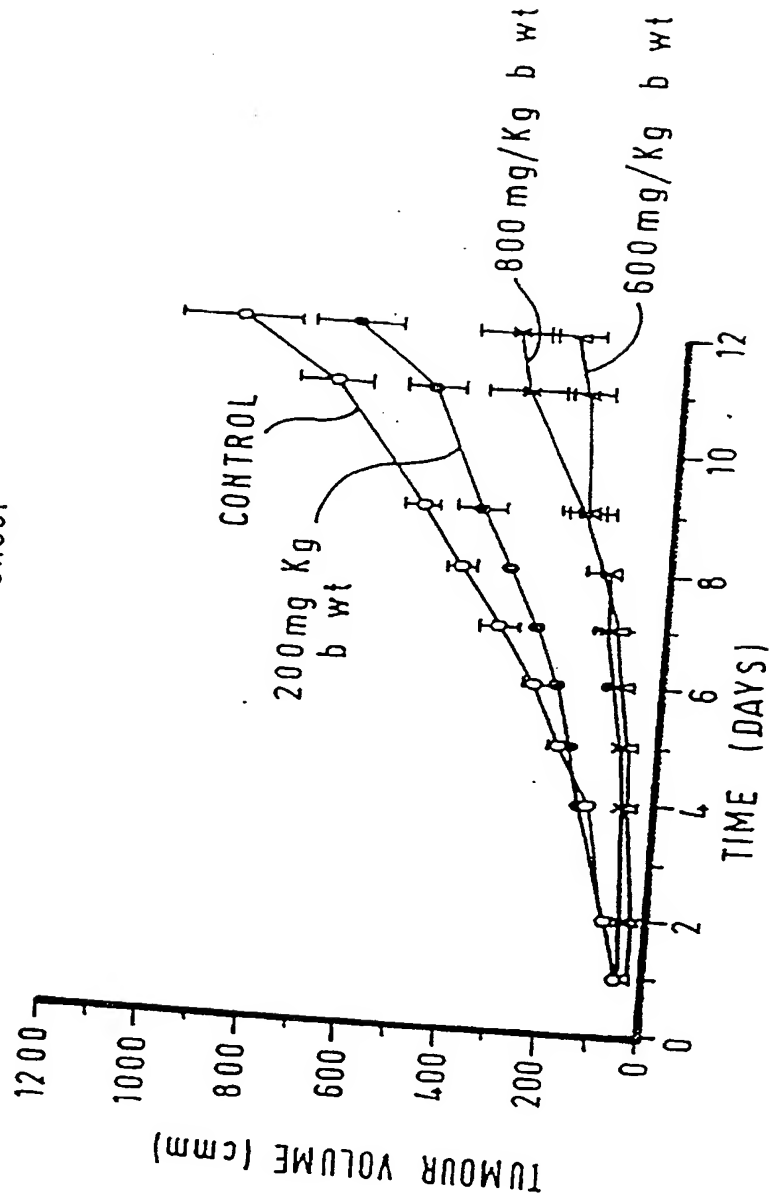


FIG. 4

THE EFFECTS OF 2,CHLORO,5,NITROBENZOIC ACID ON THE GROWTH OF MAC13 MURINE ADENOCARCINOMA COLON TRANSPLANTED SC AND DRUG ADMINISTERED IP DAILY 6 ANIMALS PER GROUP



5

ARYLATING AGENTS

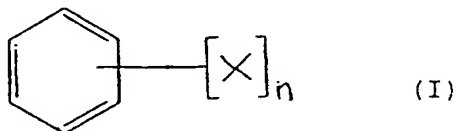
The present invention relates to arylating agents, in particular phenylating agents, which are suitable as therapeutic compounds, especially in the treatment of cancer and disease caused by viral infection.

In its broadest sense, the invention relates to arylating agents for use in the treatment of neoplasm or of viral infection such as by HIV. The arylating agent will in particular be a compound having an aryl group whose aromatic ring is preferably carbocyclic and has in any event at least one labile substituent and at least one electrophilic substituent. The carbocyclic or other aromatic ring is preferably monocyclic and in any event the aromatic ring is conveniently one which bears one or more carboxylic acid or sulphonic acid moieties together with one or more nitro and/or amino groups and/or one or more halogen substituents. The substituents preferably do not include more than two nitro substituents. A combination of halogen (eg. chloro) and nitro substituents, especially in the context of a monocyclic arylating agent comprised of a ring carrying a carboxylic acid substituent, is a particularly efficacious structure. One example of such a structure is one based on a combination of mono-nitro- and

- 5 mono-chloro- substitution (eg. 2-chloro-5-nitro benzoic acid and 2-chloro-4-nitro benzoic acid).

According to the invention there is provided a compound for use in the treatment of cancer or disease caused by viral  
 10 infection, in particular AIDS, which compound comprises an aromatic ring structure having at least one labile leaving group substituent and at least one electrophilic group substituent provided that where there are two ortho nitro  
 groups and a para sulphonic group or three symmetrical  
 15 nitro groups and the labile group at position one is a group as defined in International Specification No. WO91/15200, use is at a concentration of more than  $1 \times 10^{-3}$  moles/litre.

- 20 Generally speaking the compound of the invention may be of the general formula:



25

wherein n is an integer and is at least 2 and each X is the same or different and is a labile group or an electrophilic

5 group, provided that when there are at least two groups X which are other than nitro at least one is a labile group and at least one is an electrophilic group.

Moreover, since treatment is sought by what is believed to  
10 be an arylating mechanism use is typically at relatively high concentrations and consequently doses. Generally, such concentrations for use of the compounds of the invention will be at least about  $1 \times 10^{-2}$  moles/litre, which in dosage terms is generally at least about 5 mg/kg

15 In selecting the substituent groupings for a compound according to the invention an essential feature is the provision within any particular aromatic ring context of at least one labile group substituent and at least one  
20 electrophilic group substituent. Moreover, a group which may be classified as labile within one particular ring context may be classifiable as electrophilic within another alternative ring context. Furthermore, where there are at least two nitro substituents the labile group substituent  
25 may be a ring hydrogen.

That having been understood preferred substituent groups may be defined as those wherein at least one X is selected from each of the following groups, namely:

- 5 electrophilic groups -  $\text{SO}_3\text{H}$ ,  $\text{SO}_3\text{M}$  (where M is a metal e.g. potassium), halogen and  $\text{NO}_2$ .
- labile groups - halogen,  $\text{SO}_3\text{H}$ ,  $\text{SO}_3\text{M}$  (where M is a metal),  $\text{NH}_2$ , substituted  $\text{NH}_2$  e.g.  $\text{NHR}_1$ ,  $\text{NR}_1\text{R}_2$  (where  $\text{R}_1$ , and  $\text{R}_2$  are the same or different and are each alkyl, alkyloxy or hydroxyalkyl),  $\text{COOH}$ ,  $\text{CONH}_2$ , substituted  $\text{CONH}_2$  e.g.  $\text{CONHR}_1$ ,  $\text{CONR}_1\text{R}_2$  (where  $\text{R}_1$  and  $\text{R}_2$  are as defined above) and  $\text{COOR}_3$  (where  $\text{R}_3$  is a metal or alkyl).
- 10
- 15

Thus, as general examples of compounds of the invention there may be mentioned the following, namely:

20

chlorodinitrobenzenesulphonic acids

chlorobenzenesulphonic acids

dichlorobenzenesulphonic acids

aminodinitrobenzenesulphonic acids

25

nitromethylbenzenesulphonic acids

glutathionyldinitrobenzenesulphonic acids

nitrochlorobenzenesulphonic acids

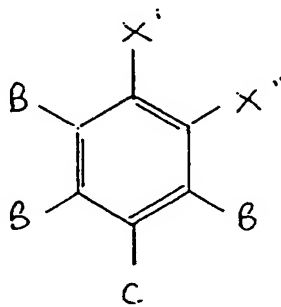
dinitrobenzenesulphonic acids



- 5        dinitrochlorobenzenes  
          dinitrofluorobenzenes  
          dichlorodinitrobenzenes  
          trinitrophenols e.g. picric acid  
          trinitroanilines
- 10       trinitrochlorobenzenes  
          trinitrobenzenesulphonic acids  
          chlorodinitrobenzoic acids  
          dichlorobenzoic acids  
          dinitrobenzoic acids
- 15       nitrochloroanisoles  
          aminodinitrobenzamides  
          dinitroanilines  
          dinitrochloroanilines  
          chloronitroanilines
- 20       dinitrofluoroanilines

The above compounds may typically be summarised by compounds of the general formula:

25



(II)



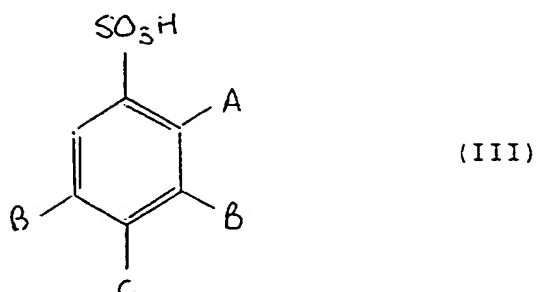
- 5        wherein X' is SO<sub>3</sub>H, SO<sub>3</sub>M (where M is a metal), halogen e.g. chloro, fluoro etc., COQ (where Q is hydroxy, amino or substituted amino, or the group OR<sub>3</sub> in which R<sub>3</sub> is a metal or alkyl), NH<sub>2</sub>, substituted NH<sub>2</sub>, NO<sub>2</sub> or OH,
- 10        X'' is hydrogen, halogen, glutathione or nitro,  
each B is the same or different and is hydrogen,  
halogen or nitro and
- 15        C is hydrogen, nitro, amino (including substituted amino), halogen, alkyl or glutathione.

In such compounds the following are preferred features:

- 20        X' is SO<sub>3</sub>H, SO<sub>3</sub>M (where M is a metal), halogen e.g. chloro, fluoro etc., amino, nitro or COOH, and
- C is hydrogen, alkyl e.g. methyl, amino or nitro.

25        The compounds which exhibit anti-cancer and anti-viral effects according to the invention may be sub-divided into a number of preferred groupings, for example, as follows:

5 (i) A compound of the general formula:



10

wherein A is hydrogen, halogen e.g. chloro,  
fluoro etc., or glutathione,

15

B is hydrogen, nitro or halogen e.g. chloro etc.,

C is hydrogen, nitro, amino (including  
substituted amino), halogen, alkyl or  
glutathione, and

20

D is hydrogen, halogen or nitro.

The above compounds of formula III are preferred because it  
is believed that the sulphonic grouping can contribute an  
emulsifying effect which is useful because it increases the  
solubility of the compounds, which in turn gives better  
bioavailability in cellular terms.

25

5 Amongst the above compounds of formula III, those more preferred are:

4-chloro-3,5-dinitrobenzenesulphonic acid  
4-chlorobenzenesulphonic acid  
10 2,5-dichlorobenzenesulphonic acid  
4-amino-3,5-dinitrobenzenesulphonic acid  
3-nitro-4-methylbenzenesulphonic acid  
2-chloro-3,5-dinitrobenzenesulphonic acid  
2-glutathionyl-3,5-dinitrobenzenesulphonic acid  
15 4-glutathionyl-3,5-dinitrobenzenesulphonic acid  
3-nitro-4-methylbenzenesulphonic acid  
3-nitro-4-chlorobenzenesulphonic acid  
2,4-dinitrobenzenesulphonic acid.

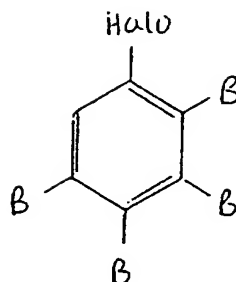
20 Especially preferred are:

4-chloro-3,5-dinitrobenzenesulphonic acid  
4-chlorobenzenesulphonic acid  
2,5-dichlorobenzenesulphonic acid  
25 4-amino-3,5-dinitrobenzenesulphonic acid  
3-nitro-4-methylbenzenesulphonic acid  
2-chloro-3,5-dinitrobenzenesulphonic acid

5

(ii) A compound of the general formula:

10



(IV)

15

wherein halo is halogen e.g. chlorine, fluorine etc., and each B is the same or different and is as defined above.

Amongst the above compounds of formula IV, those more preferred are:

20

1-chloro-2,4-dinitrobenzene

1-chloro-3,4-dinitrobenzene

1-fluoro-2,4-dinitrobenzene

1,2-chloro-4,5-dinitrobenzene

1,3-chloro-4,5-dinitrobenzene.

25

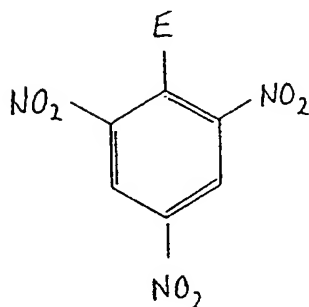
Especially preferred are:

1,3-chloro-4,5-dinitrobenzene

1-chloro-2,4-dinitrobenzene

1-fluoro-2,4-dinitrobenzene

5 (iii) A compound of the general formula:



(V)

10

wherein E is SO<sub>3</sub>H, SO<sub>3</sub>M (where M is a metal e.g. potassium), NH<sub>2</sub> or substituted NH<sub>2</sub>, halogen or hydroxy.

15

Amongst compounds of formula V, those more preferred are:

2,4,6-trinitrophenol (picric acid),

2,4,6-trinitroaniline,

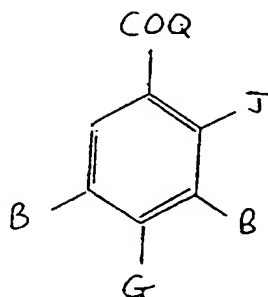
20

2,4,6-trinitrochlorobenzene.

2,4,6-trinitrobenzenesulphonic acid.

Of the above preferred compounds the first and third are especially preferred.

- 5 (iv) A compound of the general formula:



(VI)

10

wherein each B is the same or different and is as defined above,

15

G is as defined above for group C except for alkyl and glutathione,

J is hydrogen or halogen, and

20

Q is hydroxy, amino or substituted amino, or the group  $OR_3$  in which  $R_3$  is a metal or alkyl.

Amongst compounds of formula VI, those more preferred are:

25

2,4-chloro-3,5-dinitrobenzoic acid

4-chloro-3,5-dinitrobenzoic acid

2,5-dichlorobenzoic acid

2,4-dinitrobenzoic acid





5            2,4-dinitro-5-fluoroaniline

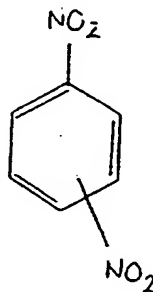
Especially preferred is:

10            2,6-dinitroaniline

As mentioned above, where there are at least two nitro substituents a ring hydrogen may provide a labile group.

Within that context there may be mentioned:

15            (vi)            A compound of the general formula:



(VIII)

20

that is to say:

1,2-dinitrobenzene  
1,3-dinitrobenzene  
25            1,4-dinitrobenzene

The compounds of the invention may be prepared by known process techniques for preparing benzene substituted compounds. Such techniques are described in various

5 standard texts, for example, "Organic Syntheses" 1963  
Collective Volume 4, pages 364 to 366, by Harry P. Schultz  
and published by John Wiley and Sons Inc.

The compounds of the invention may be formulated for use as  
10 pharmaceutical compositions (eg for iv, ip, oral or sc  
administration) comprising at least one active compound and  
a diluent or carrier. Thus, the invention includes a  
pharmaceutical composition, which composition comprises a  
compound according to the invention and a pharmaceutically-  
15 acceptable diluent or carrier (eg aqueous).

Such a composition may be in bulk form or, more preferably,  
unit dosage form. Thus, for example, the composition may  
be formulated as a tablet, capsule, powder, solution or  
20 suspension. Soft gel capsules may be especially  
convenient. The composition may be a liposomal formulation  
or administered in a slow sustained release delivery  
system.

25 Compositions in accordance with the invention may be  
prepared using the active compounds defined herein in  
accordance with conventional pharmaceutical practice. The  
diluent, excipients or carriers etc. which may be used are  
well known in the formulation art and the form chosen for

5 any particular regimen will depend on the given context and the physician's choice.

Thus, for example, as illustrated below the compounds of the invention may be administered in solution in sterile  
10 deionised water. Also, if necessary, solution may be facilitated using dimethyl sulphoxide (DMSO) or alternatively an alcohol, a glycol or a vegetable oil. The compounds are most favourably administered in corn oil or as a solution in DMSO/sterile water.

15

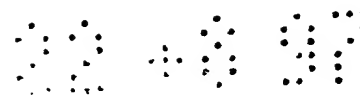
The invention further includes within the above use context the use of a compound as defined herein in the preparation of a medicament for the prophylaxis or therapy of cancer or viral infection, eg to reduce or eliminate cancerous  
20 growth.

In using a compound of the invention dosage guidance can be taken from animal studies such as that described below. In such studies doses of from about 50 mg/kg typically up to  
25 about 200 mg/kg and even up to about 400 mg/kg and beyond have proved effective. Thus it is to be expected that a typical dosage for humans will be from about 5 mg/kg typically to about 20 mg/kg and perhaps generally to about

5     40 mg/kg or higher. The concentration and dose are to be  
sufficient to bring an arylating mechanism into play.

As can be seen from the especially preferred compounds  
listed above, those compounds of the invention which are  
10    most efficacious are in believed descending order of  
activity as follows, namely:

4-chloro-3,5-dinitrobenzenesulphonic acid  
4-chlorobenzenesulphonic acid  
15    1,5-chloro-2,3-dinitrobenzene  
2,4,6-trinitrophenol (picric acid)  
2,4-chloro-3,5-dinitrobenzoic acid  
2,5-dichlorobenzenesulphonic acid  
4-amino-3,5-dinitrobenzenesulphonic acid  
20    3-nitro-4-methylbenzenesulphonic acid  
4-chloro-3,5-dinitrobenzoic acid  
2,6-dinitroaniline  
2,4-dinitrochlorobenzene  
2,4-dinitrofluorobenzene  
25    2,4,6-trinitrochlorobenzene  
2,5-dichlorobenzoic acid  
2-chloro-3,5-dinitrobenzenesulphonic acid  
2,4-dinitrobenzoic acid



5 Especially preferred compounds are those wherein at least one X is selected from:

labile substituent group(s) - 1 or 2 halogen groups  
and/or  $\text{NH}_2$  or  
10 substituted  $\text{NH}_2$  and/or  
COOH or substituted COOH  
and/or alkyl and/or  
SO<sub>3</sub>H/SO<sub>3</sub>M

electrophilic substituent  
15 group(s) - 1 or 2 nitro groups  
and/or SO<sub>3</sub>H/SO<sub>3</sub>M and/or  
1 or 2 halogen groups

Moreover, while the compounds of the invention can be used  
20 within the dosage regimen exemplified above, where there  
are three symmetrical nitro substituents or the active  
agent is otherwise as disclosed in International  
Specification No WO 91/15200, as indicated above, the  
concentration of active agent in any formulation must be  
25 more than  $1 \times 10^{-3}$  moles/litre and preferably at least  $1 \times 10^{-2}$  moles/litre.

As shown by the results reported in Table 8 below, 2-chloro-5-nitrobenzoic acid shows consideration anti-tumour

- 5 activity in vivo. This could not be supported in vitro and it appears some compounds according to the invention require activation in the patient's liver. This and some other compounds may also be immunomodulators.
- 10 The following animal study illustrates the remarkable activity of compounds of the invention.

#### ANIMAL STUDIES

- 15 The purpose of these studies was to evaluate the anti-tumour properties of a group of compounds with structural similarities that may act as arylating agents. Their in vivo anti-tumour responses were assessed against two ascitic tumours, the MAC15A murine colon adenocarcinoma and
- 20 the P388 murine leukaemia and various solid tumour models. The MAC15A ascites tumour cells were transplanted into male NMR1 mice by ip inoculation at a cell density of  $1 \times 10^5$  cells in 200ml buffer (Table 1). The P388 were transplanted ip into male BDF1 mice at cell density of  $1 \times$
- 25  $10^6$  cells in 200ml buffer (Table 2). The solid tumour models included the MAC13 and MAC16 murine colon adenocarcinomas, the B16 F1 murine melanoma and the M5076 reticulum cell sarcoma.

5 Treatment commenced 3 days after ip transplant or, in the case of solid tumours such as MAC13 and MAC16, treatment commenced when average tumour volumes reached 40mm<sup>3</sup>.

The animals were located in both cases into groups of 5 to 10 8 animals.

The animals were sacrificed after 12 days or when tumours ulcerated, tumour volume exceeded 1000mm<sup>3</sup> or loss of body weight exceeded 50%.

15 Except where otherwise stated, the compounds used were dissolved in DMSO and diluted in sterile distilled water, at appropriate concentrations before administration in a solvent volume of 200 ml. Anti-tumour responses were  
20 obtained by comparing the median survival times or tumour growth inhibition against solvent controls. The results obtained are as shown in Tables 1 to 8 below.

. Preparation of dosage solutions is exemplified as follows:-

25

Subjects:                      No        :    10 animals

   Weight:    22g

Dosage: 50mg/kg body weight per animal per day  
30 thus 1.1mg per mouse per day

5

Total Mass Dosage: 55mg active ingredient (referred to 5 day treatment regime)

10

Total Formulation: 10ml solvent plus 55mg for division into 50 doses of 1.1mg dissolved in 200µl solvent

T/C% is determined as follows:-

15

Animal Survival  $\frac{\text{Test}}{\text{T days}}$        $\frac{\text{Control}}{\text{C days}}$

20

$$T/C\% = \frac{T}{C} \times 100$$

Example

25

Animal Survival  $\frac{\text{Test}}{443 \text{ days}}$        $\frac{\text{Control}}{100 \text{ days}}$

30

$$T/C\% = \frac{443}{100} \times 100 = 443$$

A figure of 158 or above indicates performance justifying clinical trial.

35

Conclusions

The effect of a group of primarily halogenated arylating compounds on the growth rate of a number of experimental



5     tumours has been evaluated *in vivo* and the following findings were noted:

1.     Structure-activity relationships against the MAC15A murine colon adenocarcinoma, in the female NMRI mice  
10     showed maximal activity on a split-dose schedule and when the halogen was maximally activated, for nucleophilic attack.

2.     The most active compound was 4-chlorobenzenesulphonic  
15     acid (T/C<sub>50</sub> 443) administered at 100 mg/kg body weight in a daily schedule of 5 days.

3.     Against the M5076 reticulum cell sarcoma, 2,4-dichloro-3,5-dinitrobenzoic acid showed activity on a  
20     split-dose schedule down to 25 mg/kg body weight by both ip and sc routes. Both the amide and the methyl ester showed 10-fold increase in toxicity and were without antitumour activity. The acid also effectively inhibited growth of B16 murine melanoma and the MAC16 murine colon  
25     adenocarcinoma.

It is concluded that this group of compounds show a wide spectrum of activity against murine models.

5

**TABLE 1**

Anti-tumour activity against MAC15A (murine adenocarcinoma colon). Structure-Activity relationship. 5 animals per group. Dose 100 mg kg<sup>-1</sup> ip per day.

10

|    | Compound                                       | Schedule<br>(days) | T/C% <sup>a</sup> |
|----|--|--------------------|-------------------|
| 15 | 4-chlorobenzenesulfonic acid                   | 1,2,3,4,5          | 443               |
|    | 4-chloro-3,5-dinitrobenzenesulfonic acid       | 1,2,3,4,5          | 414               |
|    | 1,5-dichloro-2,3-dinitrobenzene                | 1,2,3,4,5          | 386               |
|    | 2,4,6-trinitrophenol                           | 1,2,3              | 300               |
| 20 | 4-amino-3,5-dinitrobenzenesulfonic acid        | 1,2,3,4,5          | 286               |
|    | 4-chloro-3,5-dinitrobenzoic acid               | 1,2,3,4,5          | 271               |
|    | 2,4-dichloro-3,5-dinitrobenzoic acid           | 1,2                | 243               |
|    | 2-glutathionyl-3,5-dinitrobenzenesulfonic acid | 1,2,3,4,5          | 242               |
|    | 3-nitro-4-methylbenzenesulfonic acid           | 1,2,3,4,5          | 229               |
| 25 | 2,6-dinitroaniline                             | 1,2,3,4,5          | 214               |
|    | 2,5-dichlorobenzenesulfonic acid               | 1,2,3,4,5          | 212               |
|    | 1,4-dinitrobenzene                             | 1,2                | 200               |
|    | 1-chloro-3,4-dinitrobenzene                    | 1,2,3,4,5          | 200               |
|    | 1-chloro-2,4-dinitrobenzene                    | 1,2,3,4,5          | 188               |
| 30 | 2,4,6-trinitrobenzenesulfonic acid             | 1,2,3,4,5          | 188               |
|    | 2-chloro-4-nitroaniline                        | 1,2,3,4,5          | 171               |
|    | 2,5-dichlorobenzoic acid                       | 1,2,3,4,5          | 171               |
|    | 2,4-dinitrobenzenesulfonic acid                | 1,2,3,4,5          | 171               |
|    | 1,2-dichloro-4,5-dinitrobenzene                | 1,2,3,4,5          | 171               |
| 35 | 4-chloro-3-nitrobenzenesulfonic acid           | 1,2,3,4,5          | 140               |
|    | 2-chloro-3,5-dinitrobenzenesulfonic acid       | 1,2,3,4,5          | 137               |
|    | 1-chloro-2,4,6-trinitrobenzene                 | 1,2,3              | 113               |
|    | 4-glutathionyl-3,5-dinitrobenzene              | 1,2,3,4            | 113               |
|    | 2,4-dinitroaniline                             | 1,2                | 100               |
| 40 | 2,4-dinitrobenzoic acid                        | 1,2,3,4,5          | 100               |
|    | 3,5-dinitrobenzoic acid                        | 1,2,3,4,5          | 100               |
|    | 4-amino-3,5-dinitrobenzamide                   | 1                  | 100               |
|    | 4-chloro-3-nitroanisole                        | 1,2,3,4,5          | 100               |
|    | 4-chloro-2,6-dinitroaniline                    | 1,2,3,4,5          | 87                |
| 45 | 6-chloro-2,4-dinitroaniline                    | 1,2,3,4,5          | 87                |
|    | 1-fluoro-2,4-dinitroaniline                    | 1                  | 75                |
|    | 1-flouro-2,4-dinitrobenzene                    | 1                  | 62.5 <sup>b</sup> |

50

a=median, T-test group, C-solvent control; b-toxic death

5

TABLE 2

Anti-tumour activity against P388 (murine leukaemia).  
Eight animals per group. IP treatment on day 1 to 5.  
Dosage is per day.

10

| <u>Compound</u>                                   | <u>Dose</u>            | <u>TC%<sup>a</sup></u> |
|---|------------------------|------------------------|
| 4-chloro-3,5-dinitrobenzene-<br>sulphonic acid    | 100mg kg <sup>-1</sup> | 203                    |
| 15 4-chloro-3,5-dinitrobenzene-<br>sulphonic acid | 50 mg kg <sup>-1</sup> | 259                    |

a=mean, T=test group, C=solvent control.

20

TABLE 3

Anti-tumour activity against P388 (murine leukaemia)  
treated ip with 4-chloro-3,5-dinitrobenzenesulfonic acid  
(CDNSA). 8 animals per group. Dosage is per day.

25

| <u>Compound</u> | <u>Dose (mg/kg)</u> | <u>Schedule (days)</u> | <u>T/C%<sup>a</sup></u> |
|-----------------|---------------------|------------------------|-------------------------|
| 30 CDNSA        | 100                 | 1,2,3,4,5              | 2 2 5                   |
|                 | 75                  | 1,2,3,4,5              | 3 0 0                   |

35

a=mean, T-test group, C-solvent control

40

5

**TABLE 4**

Anti-tumour activity against M5076-reticulum cell sarcoma  
16 days after im transplant. 7 animals per group. Drugs  
dissolved in corn oil. Dosage is per day.

10

15

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25

30

| Compound | Dose<br>(mg/kg)  | Route | Schedule<br>(days) | % Tumour Weight<br>Inhibition |
|----------|------------------|-------|--------------------|-------------------------------|
| 2,4 BA   | 75 <sup>a</sup>  | ip    | 1,4,6,9            | 79,88 <sup>b</sup>            |
|          | 50               | ip    | 1,4,6,9            | 57                            |
|          | 25               | ip    | 1,2,4,6,9          | 75                            |
|          | 75               | sc    | 1,4,5,7,9          | 66                            |
|          | 50               | sc    | 1,2,4,5,6,7,9      | 76                            |
|          | 25               | sc    | 1,2,4,5,6,7,9      | 63                            |
| 2,4 BZ   | 2.5 <sup>a</sup> | ip    | 1,2,3,4,5,6,7,8,9  | 51                            |
|          | 1.25             | ip    | 1,2,3,4,5,6,7,8,9  | 34                            |
| 2,4 BM   | 1.0 <sup>a</sup> | ip    | 1,2,3,4,5,6,7,8,9  | 41                            |
|          | 0.5              | ip    | 1,2,3,4,5,6,7,8,9  | 39                            |
|          | 0.25             | ip    | 1,2,3,4,5,6,7,8,9  | 42                            |

a = Maximum tolerated dose

b = two independent experiments; 4 animals had no tumour in  
the second experiment

35

2,4 BA = 2,4-dichloro-3,5-dinitrobenzoic acid

2,4 BZ = 2,4-dichloro-3,5-dinitrobenzamide

40

2,4 BM = 2,4-dichloro-3,5-dinitrobenzoic acid methyl ester

% Tumour Weight Inhibition:-

45

Treated

Control

Agm

Bgm

Tumour weight

50

% inhibition =  $\frac{B - A}{B} \times 100$

5

**TABLE 5**

Anti-tumour activity against B16F1-murine melanoma 12 days after sc transplant. 6 animals per group. Drugs dissolved in corn oil. Dosage is per day.

10

15

20

25

30

35

| Compound | Dose (mg/kg)     | Route | Schedule (days) | % Tumour Weight Inhibition |
|----------|------------------|-------|-----------------|----------------------------|
| 2,4 BA   | 75 <sup>a</sup>  | ip    | 1,5             | 71,81 <sup>b</sup>         |
|          | 50               | ip    | 1,5             | 45,56 <sup>b</sup>         |
|          | 25               | ip    | 1,5             | 13                         |
|          | 75               | sc    | 1,3,5           | 30                         |
|          | 50               | sc    | 1,3,5           | 9                          |
|          | 25               | sc    | 1,3,5           | 22                         |
| 2,4 BZ   | 2.5 <sup>a</sup> | ip    | 1,2             | 39                         |
|          | 1.25             | ip    | 1,2             | 17                         |
| 4 BA     | 100              | ip    | 1,5             | 39                         |
|          | 75               | ip    | 1,5             | 41                         |
|          | 50               | ip    | 1,5             | 10                         |
| 4 BZ     | 5 <sup>a</sup>   | ip    | 1,3,5           | 18                         |
|          | 2.5              | ip    | 1,3,5           | 18                         |
|          | 1.25             | ip    | 1,3,5           | 27                         |
| 4BM      | 2.5 <sup>a</sup> | ip    | 1,3             | 67                         |
|          | 1.25             | ip    | 1,2,3           | 43                         |

a = Maximum tolerated dose

b = Two independent experiments

45

2,4 BA = 2,4-dichloro-3,5-dinitrobenzoic acid

2,4 BZ = 2,4-dichloro-3,5-dinitrobenzamide

4 BA = 4-chloro-3,5-dinitrobenzoic acid

4 BZ = 4-chloro-3,5-dinitrobenzamide

4 BM = 4-chloro-3,5-dinitrobenzoic acid methyl ester

5

TABLE 6

Anti-tumour activity against MAC13 murine colon  
adenocarcinoma 12 days after im transplant. Drugs  
dissolved in corn oil. Dosage is per day.

10

| Compound | Dose<br>(mg/kg)    | Route | Schedule<br>(days) | % Tumour Weight<br>Inhibition |
|----------|--------------------|-------|--------------------|-------------------------------|
| 2,4 BA   | 75 <sup>a</sup>    | ip    | 1,4,5              | 45                            |
| 2,4 BA   | 50                 | ip    | 1,2,3,4,5,6,7,8,9  | 39                            |
| 2,4 BA   | graph <sup>3</sup> | ip    | graph <sup>3</sup> | graph <sup>3</sup>            |
| 2,4 BZ   | 2.5 <sup>a</sup>   | ip    | 1,2,3,4,5,6,7,8,9  | 51                            |
| 2,4 BZ   | 1.25               | ip    | 1,2,3,4,5,6,7,8,9  | 17                            |
| 2 BA     | graph <sup>4</sup> | ip    | graph <sup>4</sup> | graph <sup>4</sup>            |

25

a = maximum tolerated dose

2,4 BA = 2,4-dichloro-3,5-dinitrobenzoic acid<sup>3</sup>

2,4 BZ = 2,4-dichloro-3,5-dinitrobenzamide

30

2 BA = 2-chloro-5-nitrobenzoic acid

(3: see Figure 3 of the drawings; 4: see Figure 4 of the drawings)

5

TABLE 7

10 Anti-tumour activity against MAC16, murine colon adenocarcinoma sc transplant on day 11 after the beginning of treatment with 2,4-dichloro-3,5-dinitrobenzoic acid (2,4 BA). Drug dissolved in corn oil. The tumour volumes were at least 40mm<sup>3</sup> at the beginning of the treatment. 6 animals per group. Dosage is per day.

15

| Compound | Dose<br>(mg/kg) | Route | Schedule<br>(days) | % Tumour Weight<br>Inhibition |
|----------|-----------------|-------|--------------------|-------------------------------|
|          |                 |       |                    |                               |
| 2,4 BA   | 75 <sup>a</sup> | ip    | 1,2,5,8            | 88                            |
|          | 50              | ip    | 1,2,4,5,8          | 91                            |

20

a = maximum tolerated dose

5

TABLE 8

10 Anti-tumour activity against B16 murine melanoma 12 days after sc transplant on female C57/black mice. 6 animals per group. Dosage is per day and is ip.

| 15 | Compound                     | Dose (mg/kg) | Schedule (days) | Tumour Weight Inhibition |
|----|------------------------------|--------------|-----------------|--------------------------|
| 20 | 2-chloro-5-nitrobenzoic acid | 700          | 1,2,3,4,5,6     | 62                       |

25 In addition, the following primary assay was used to investigate the anti-viral activity of compounds in accordance with the invention, in particular 4-chloro-3,5-dinitrobenzenesulphonic acid.

Anti-tumour activity and toxicity studies have additionally been completed for the following compounds with broadly satisfactory results:-

30

C22 2,5-dichloro-4-nitrobenzoic acid

C23 2,4-dichloro-5-nitrobenzoic acid

C24 2,6-dichloro-4-nitrobenzoic acid

C25 2-amino-5-nitrobenzoic acid

35 C26 2-hydroxy-5-nitrobenzoic acid

C27 3,5-dichloro-4-nitrobenzoic acid



5

PRIMARY ASSAY

(i) *Acute Infection Assay.* High titre virus stocks of the human immunodeficiency virus HIV-1<sub>RF</sub> were grown in H9  
10 cells with RPMI 1640 (Flow laboratories) supplemented with 10% fetal calf serum, penicillin (100IU/ml). Cell debris was removed by low speed centrifugation, and the supernatant stored at -70°C until required. In a typical assay C8166 T-lymphoblastoid CD4+ cells were incubated with  
15 10xTCID50 HIV-1<sub>RF</sub> at 37°C for 90 minutes and then washed three times with phosphate buffered saline (PBS). Cell aliquots ( $2 \times 10^5$ ) were resuspended in 1.5 ml growth medium in 6 ml tubes, and compounds in log dilutions [200mM to 0.2mM] were added immediately. 20 mM stock solutions of  
20 each compound were made up in 70% alcohol. The compounds were stored as a powder and made up freshly in distilled water before each experiment or were stored as a 20 mM stock solution in 70% alcohol. The final concentration of alcohol in the tissue culture medium was 1%. The cells  
25 were then incubated at 37°C in 5% CO<sub>2</sub>. At 72 hours post-infection 200 ml of supernatant was taken from each culture and assayed for HIV (Kingchington et al, 1989, Robert et al 1990) using an antigen capture ELISA which recognizes all the core proteins equally (Coulter Electronics, Luton, UK).

5 The following controls were used: supernatants taken from  
uninfected and infected cells, infected cells treated with  
AZT (Roche Products UK, Ltd) and ddC (Roche) and R031-8959  
(Roche) an inhibitor of HIV proteinase. The IC<sub>50</sub> activities  
of 8959, AZT and ddC in infected cells were 1, 10, 20 nM  
10 and 200 nM respectively (accompanying Figure 2). The ELISA  
plates were read with a spectrophotometer. Compounds were  
tested in duplicate at each concentration, and the data  
shown is the average of at least two assays. This assay  
assesses the activity of compounds by measuring their  
15 inhibition of HIV core antigen levels.

(ii) *Chronically Infected Cell Assay.* Chronically  
infected cells (H9rf) were washed three times to remove  
extracellular virus and incubated with the active compounds  
20 (200-0.2 mM) for four days. HIV-1 antigen in the  
supernatant was then measured using an ELISA.

To test for compound toxicity uninfected H9 cells were  
incubated with the compounds for four days. Supernatants  
25 were discarded and the cells resuspended in 200ml pg growth  
medium containing <sup>14</sup>C protein hydrolysate. After 6 hours  
the cells were harvested and the <sup>14</sup>C incorporation measured.

5

(iii) Toxicity Assay. To test for compound toxicity, aliquots of  $2 \times 10^5$  of uninfected cells were cultured with the compounds in the same dilutions for 72 hours. The cells were then washed with PBSA and resuspended in 200ml of growth medium containing  $^{14}\text{C}$  protein hydrolysate. After 12 hours the cells were harvested and the  $^{14}\text{C}$  incorporation measured. Uninfected, untreated cells were used as controls. Toxicity is expressed as inhibition of uptake of  $^{14}\text{C}$  protein hydrolysate.

15

The results of these assays for 4-chloro-3,5-dinitrobenzenesulphonic acid are shown in accompanying Figure 1 in which RC stands for Radopath compound C i.e. 4-chloro-3,5-dinitrobenzenesulphonic acid. The results are also summarised in Table 9 below:

TABLE 9

|    |  |                        |                        |           |
|----|--|------------------------|------------------------|-----------|
| 25 | <u>Compound</u>                                    | <u>IC<sub>50</sub></u> | <u>CD<sub>50</sub></u> | <u>TI</u> |
|    | 4-chloro-3,5<br>-dinitrobenzene-<br>sulphonic acid | 3mM                    | 80mM                   | 28.6      |

5

The  $IC_{50}$  is the drug concentration that causes a 50% reduction in HIV core antigen levels as detected by the Coulter P24 antigen assay and is determined by doubling dilutions of supernatant taken from tubes containing untreated acutely infected cells. The  $CD_{50}$  is the concentration of drug that causes a 50% inhibition of cells as measured by  $^{14}C$  protein hydrolysate uptake. The therapeutic index (TI) is determined by dividing the  $CD_{50}$  by the  $IC_{50}$ .

15

Further results for other compounds in accordance with the invention are summarised in Table 10 below:

5

TABLE 10

|    | <u>Compound</u>                                | <u>IC<sub>50</sub></u> | <u>CD<sub>50</sub></u> | <u>TI</u> |
|----|--|------------------------|------------------------|-----------|
| 10 | 2-chloro-3,5-dinitro-<br>benzenesulphonic acid | 25mm                   | >200mm                 | >8        |
| 15 | 4-amino-3,5-dinitro-<br>benzenesulphonic acid  | 20mm                   | 100mm                  | 5         |
|    | 2,4,6-trinitrophenol                           | <0.2mm                 | 95mm                   | >475      |
| 20 | 4-chloro-3,5-dinitro-<br>benzoic acid          | 30mm                   | 70mm                   | 2.33      |

Initial tests performed approximately contemporaneously indicated 2-chloro-5-nitrobenzoic acid would demonstrate performance at least as efficacious, if not more so, as any  
 25 of the compounds whose tests are reported herein.

Following the methodology set forth earlier for performance assay against HIV, more extensive assays were performed as reported in Tables 11 below:

5

TABLE 11.1

## STRUCTURE-ACTIVITY RELATIONSHIP AGAINST HIV VIRUS

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| CODE    | COMPOUNDS   | $^{50}\text{IC}_{50}$ | $^{50}\text{CC}_{50}$ |
|---------|---|-----------------------|-----------------------|
| GROUP A |   |                       |                       |
| P1      | picryl chloride                                   |                       |                       |
| P2      | picric acid                                       |                       |                       |
| P3      | picrylsulfonic acid (sodium salt)                 |                       |                       |
| GROUP B |   |                       |                       |
| C1      | 2,4-dichloro-3,5-dinitrobenzoic acid              |                       |                       |
| C2      | 2,4-dichloro-3,5-dinitrobenzamide                 |                       |                       |
| C3      | 2,4-dichloro-3,5-dinitrobenzoic acid methyl ester |                       |                       |
| C4      | 4-chloro-3,5-dinitrobenzoic acid                  |                       |                       |
| C5      | 4-chloro-3,5-dinitrobenzamide                     |                       |                       |
| C6      | 4-chloro-3,5-dinitrobenzoic acid methyl ester     |                       |                       |
| C7      | 2-chloro-3,5-dinitrobenzoic acid                  |                       |                       |
| C8      | 2-chloro-3,5-dinitrobenzoic acid methyl ester     |                       |                       |
| C9      | 4-chloro-3-nitrobenzoic acid                      |                       |                       |
| C10     | 2-chloro-4-nitrobenzoic acid                      |                       |                       |
| C11     | 3,4-dichlorobenzoic acid                          |                       |                       |
| C12     | 2,5-dichlorobenzoic acid                          |                       |                       |
| C13     | 4-chlorobenzoic acid                              |                       |                       |
| GROUP C |   |                       |                       |
| S1      | 4-chloro-3,5-dinitrobenzenesulfonic acid          |                       |                       |
| S2      | 2-chloro-3,5-dinitrobenzenesulfonic acid          |                       |                       |
| S3      | 4-amino-3,5-dinitrobenzenesulfonic acid           |                       |                       |
| S4      | 4-chloro-3-nitrobenzenesulfonic acid              |                       |                       |
| S5      | 4-chlorobenzenesulfonic acid                      |                       |                       |
| S6      | 4-nitrobenzenesulfonic acid                       |                       |                       |
| S7      | 2,5-dichlorobenzenesulfonic acid                  |                       |                       |
| S8      | 2,4-dinitrobenzenesulfonic acid                   |                       |                       |

5

**TABLE 11.1 (CONT/D)**

| GROUP D |                                 |
|---------|---------------------------------|
| 10      |                                 |
| E1      | 1-chloro-3,4-dinitrobenzene     |
| E2      | 1-chloro-2,4-dinitrobenzene     |
| E3      | 1,2-dichloro-4,5-dinitrobenzene |
| E4      | 2,3-dichloronitrobenzene        |
| 15      |                                 |
| E5      | 2,4-dichloronitrobenzene        |
| E6      | 2,5-dichloronitrobenzene        |
| E7      | 3,4-dichloronitrobenzene        |
| E8      | 3,5-dichloronitrobenzene        |
| E9      | 1,5-dichloro-2,3-dinitrobenzene |
| 20      |                                 |
| E10     | 1,2,3-trichloro-4-nitrobenzene  |
| E11     | 1,2,4-trichloro-5-nitrobenzene  |
| E12     | 2,4,6-trichlorobenzene          |
| E13     | 2,3,4,6-tetrachloronitrobenzene |
| E14     | pentachloronitrobenzene         |
| 25      |                                 |

**TABLE 11.2**

| P-Compounds              | IC50<br>(Antiviral) | CC50<br>(Toxicity) | SI<br>(Selectivity Index) |
|--------------------------|---------------------|--------------------|---------------------------|
| <u>Against HIV-1RF</u>   |                     |                    |                           |
| P1                       | 0.6                 | 7                  | 10                        |
| 35                       | -                   | 5                  | -                         |
|                          | 0.4                 | -                  | -                         |
| <b>Average</b>           | <b>0.5</b>          | <b>6</b>           | <b>12</b>                 |
| P2                       | 38                  | 67                 | 2                         |
| 40                       |                     |                    |                           |
| P3                       | >200                | >200               | -                         |
| <u>Against HIV-1IIIB</u> |                     |                    |                           |
| 45                       |                     |                    |                           |
| P1                       | 0.6                 | 7                  | 11.6                      |
|                          | 1                   | 7                  | 7                         |
| <b>Average</b>           | <b>0.8</b>          | <b>7</b>           | <b>9</b>                  |

22-8-97

36

5

Against chronically infected cells

|    |                |            |            |          |
|----|----------------|------------|------------|----------|
|    | P1             | 0.9        | 7          | 8        |
|    |                | 2          | 12         | 6        |
| 10 | <b>Average</b> | <b>1.5</b> | <b>9.5</b> | <b>6</b> |



5

**TABLE 11.3**

|    | C-Compounds                               | IC50<br>(Antiviral) | CC50<br>(Toxicity) | SI<br>(Selectivity Index) |
|----|---|---------------------|--------------------|---------------------------|
| 10 | <u>Against HIV-IIIB</u>                   |                     |                    |                           |
|    | C1  | 5                   | 70                 | 14                        |
|    |   | 36                  | 70                 | 2                         |
|    |   | 33                  | 70                 | 2                         |
| 15 |   | 35                  | 60                 | 2                         |
|    | <b>Average</b>                            | <b>27</b>           | <b>70</b>          | <b>3</b>                  |
|    | <u>Against HIV-1RF</u>                    |                     |                    |                           |
| 20 | C1  | 7                   | 60                 | 8.5                       |
|    |   | -                   | -                  | 56                        |
|    |   | 16                  | 56                 | 3.5                       |
|    | <b>Average</b>                            | <b>11.5</b>         | <b>57</b>          | <b>5</b>                  |
| 25 | <u>Against chronically infected cells</u> |                     |                    |                           |
|    | C1  | 16                  | 30                 | 2                         |
|    |   | 16                  | 95                 | 6                         |
|    | <b>Average</b>                            | <b>16</b>           | <b>63</b>          | <b>4</b>                  |
| 30 | <u>Against HIV-1IIIB</u>                  |                     |                    |                           |
|    | C2  | ...2                | 70                 | 35                        |
|    | C3  | 0.3                 | 7                  | 23                        |
| 35 | C4  | 40                  | 100                | 2.5                       |
|    |   | 30                  | 70                 | 2.3                       |
|    | <b>Average</b>                            | <b>35</b>           | <b>85</b>          | <b>2.4</b>                |
|    | C5  | 5                   | 50                 | 10                        |
| 40 | C6  | 5                   | 60                 | 12                        |
|    | C7  | 23                  | 150                | 6                         |
|    |   | 5                   | >200               | >10                       |
|    | <b>Average</b>                            | <b>22</b>           | <b>&gt;175</b>     | <b>8</b>                  |
| 45 | C8  | 10                  | 60                 | 5                         |
|    | C9  | >200                | >200               | -                         |

20-09

|   |      |      |      |   |
|---|------|------|------|---|
| 5 | C-10 | >200 | >200 | - |
|   | C-11 | >200 | >200 | - |
|   | C-12 | >200 | >200 | - |

10

**TABLE 11.4**

|    |             |                     |                    |                           |
|----|-------------|---------------------|--------------------|---------------------------|
| 15 | S-Compounds | IC50<br>(Antiviral) | CC50<br>(Toxicity) | SI<br>(Selectivity Index) |
|----|-------------|---------------------|--------------------|---------------------------|

|    |                        |           |           |            |
|----|------------------------|-----------|-----------|------------|
| 20 | <u>Against HIV-1RF</u> |           |           |            |
|    | S1                     | 20        | 100       | 5          |
|    |                        | 19        | 60        | 3          |
|    | <b>Average</b>         | <b>20</b> | <b>80</b> | <b>4</b>   |
| 25 | S2                     | NR        |           |            |
|    | S3                     | NR        |           |            |
| 30 | S4                     | >200      | >200      | -          |
|    | S5                     | >200      | >200      | -          |
|    | S6                     | >200      | >200      | -          |
| 35 | S7                     | >200      | >200      | -          |
|    | S8                     | 40        | 100       | 2.5        |
|    |                        | 30        | 70        | 2          |
|    | <b>Average</b>         | <b>35</b> | <b>75</b> | <b>2.4</b> |
| 40 |                        |           |           |            |

5

TABLE 11.5

|    | E-Compounds            | IC50<br>(Antiviral) | CC50<br>(Toxicity) | SI<br>(Selectivity Index) |
|----|------------------------|---------------------|--------------------|---------------------------|
| 10 | <u>Against HIV-1RF</u> |                     |                    |                           |
|    | E1                     | 4                   | 10                 | 2.5                       |
| 15 | E2                     | 4                   | 13                 | 3                         |
|    | E3                     | 4                   | 7                  | 1.5                       |
| 20 | E4                     | 80                  | >200               | 1.5                       |
|    | E5                     | 180                 | >200               | 1                         |
|    | E6                     | 110                 | >200               | 2                         |
| 25 | E7                     | >200                | >200               | -                         |
|    | E8                     | 120                 | >200               | 1.5                       |
| 30 | E9                     | ND                  |                    |                           |
|    | E10                    | >200                | 90                 | -                         |
|    | E11                    | >200                | >200               | -                         |
| 35 | E12                    | >200                | >200               | -                         |
|    | E13                    | >200                | 80                 | -                         |
| 40 | E14                    | >200                | >200               | -                         |

While the invention has been described above in various  
 45 specific details, it will be appreciated that numerous and  
 various modifications may be made within the spirit and  
 scope of the claims which follow. Thus, for example, the

- 5 functional groups can be in various other positions, of which the above specifically recited are examples only.

5

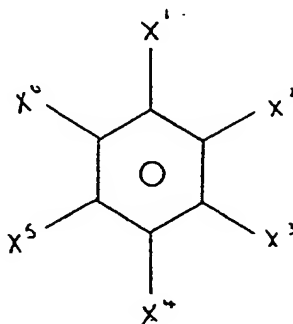
CLAIMS

1. A compound for use as a pharmaceutical, the compound comprising an aromatic ring structure having at least one labile leaving moiety and at least one electrophilic moiety.

10

2. A compound as claimed in Claim 1 and having the general formula:

15



I

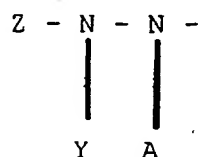
wherein one of  $X^1$  to  $X^6$  is a labile leaving moiety, one of the balance thereof is an electrophilic moiety and the remainder are the same or different and are hydrogen or a substituent.

20

3. A compound as claimed in Claim 2 wherein  $X^1$  is a labile leaving moiety, one of  $X^2$  to  $X^6$  is an electrophilic moiety and the remainder are, each independently, hydrogen or a substituent, provided that when  $X^2$  and  $X^6$  are nitro groups,  $X^4$  is neither a nitro group, a sulphonic acid group nor a sulphonate group or  $X^1$  is not a labile group as

25

5 defined below, namely a hydroxy group, an amino group, a sulfo group, a carboxy group, a methyloxy group, halogen or a hydrazyl group of the formula:



10

wherein A is hydrogen or an unpaired electron of the nitrogen atom, Y is hydrogen or an organic group and Z is an organic group, or Y and Z together with the adjacent nitrogen atom form a nitrogen-containing heterocycle.

15

4. A compound as claimed in Claim 2 wherein one of  $X^1$  to  $X^6$  is a labile leaving moiety, one of the balance thereof is an electrophilic moiety, and the remainder are the same or different and are hydrogen or an substituent with at least  
20 two thereof being other than nitro, at least one being a labile moiety and at least one being an electrophilic moiety.

5. A compound as claimed in any one of Claims 2 to 4,  
25 wherein at least one of  $X^1$  to  $X^6$  is an electrophilic moiety or labile moiety selected from the following:-

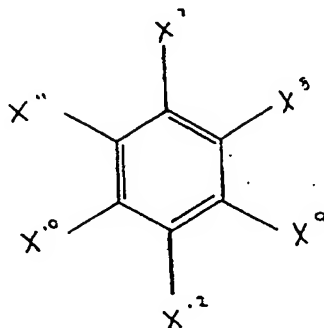
electrophilic moieties -  $\text{SO}_3\text{H}$ ,  $\text{SO}_3\text{M}$  (where M is a metal),

halogen and  $\text{NO}_2$

- 5 labile moieties - halogen,  $\text{SO}_3\text{H}$ ,  $\text{SO}_3\text{M}$  (where M is a metal), optionally substituted  $\text{NH}_2$ ,  $\text{COOH}$ , optionally substituted  $\text{CONH}_2$  and  $\text{COOR}_3$  (where  $\text{R}_3$  is a metal or alkyl).

10

6. A compound as claimed in any preceding claim which has the general formula:



II

15

wherein:-

- 20  $\text{X}^7$  is  $\text{SO}_3\text{H}$ ,  $\text{SO}_3\text{M}$  (where M is a metal), halogen,  $\text{COQ}$  (where Q is hydroxy, amino or substituted amino, or the group  $\text{OR}_3$  in which  $\text{R}_3$  is a metal or alkyl),  $\text{NH}_2$ , substituted  $\text{NH}_2$ ,  $\text{NO}_2$  or  $\text{OH}$ ;

- 25  $\text{X}^8$  is hydrogen, halogen, glutathione or nitro;

$\text{X}^9$ ,  $\text{X}^{10}$  and  $\text{X}^{11}$  are, each independently, hydrogen, halogen or nitro; and

5  $X^{12}$  is hydrogen, nitro, optionally substituted amino, halogen, alkyl or glutathione.

7. A compound as claimed in Claim 6 wherein:-

10  $X^7$  is  $SO_3H$ ;

$X^8$  is hydrogen, halogen or glutathione;

$X^9$  and  $X^{10}$  are, each independently, hydrogen, halogen or nitro;

15

$X^{11}$  is hydrogen; and

$X^{12}$  is hydrogen, nitro, optionally substituted amino, halogen, alkyl or glutathione.

20

8. A compound as claimed in Claim 7 and as set forth by name below:-

8.1 4-chloro-3,5-dinitrobenzenesulphonic acid

25 8.2 4-chlorobenzenesulphonic acid

8.3 2,5-dichlorobenzenesulphonic acid

8.4 4-amino-3,5-dinitrobenzenesulphonic acid

8.5 3-nitro-4-methylbenzenesulphonic acid



- 5     8.6   2-chloro-3,5-dinitrobenzenesulphonic acid  
      8.7   2-glutathionyl-3,5-dinitrobenzenesulphonic acid  
      8.8   4-glutathionyl-3,5-dinitrobenzenesulphonic acid  
      8.9   3-nitro-4-methylbenzenesulphonic acid  
      8.10  3-nitro-4-chlorobenzenesulphonic acid  
10    8.11  2,4-dinitrobenzenesulphonic acid  
      8.12  4-chloro-3,5-dinitrobenzene sulfonic acid  
      8.13  a salt of any of the acids listed as 8.1 and 8.12

9.    A compound as claimed in Claim 6 wherein:-

15

$X^7$  is halogen;

$X^8$ ,  $X^9$ ,  $X^{10}$  and  $X^{12}$  are, each independently, hydrogen, halogen or nitro; and

20     $X^{11}$  is hydrogen.

10.   A compound as claimed in Claim 9 and as set forth by name below:-

- 25    10.1  2,4-dinitrochlorobenzene  
      10.2  3,4-dinitrochlorobenzene  
      10.3  2,4-dinitrofluorobenzene  
      10.4  1,2-dichloro-4,5-dinitrobenzene  
      10.5  1,3-dichloro-4,5-dinitrobenzene

5 10.6 1,5-dichloro-2,3-dinitrobenzene

11. A compound as claimed in Claim 6 wherein:-

10 X<sup>7</sup> is SO<sub>3</sub>H, SO<sub>3</sub>M (where M is a metal), NH<sub>2</sub> or substituted  
NH<sub>2</sub>, halogen or hydroxy;

X<sup>8</sup> is nitro;

15 X<sup>9</sup> is hydrogen;

X<sup>10</sup> is hydrogen;

X<sup>11</sup> is nitro; and

20 X<sup>12</sup> is nitro.

12. A compound as claimed in Claim 11 and as set forth by  
name below:-

25 12.1 2,4,6-trinitrophenol (picric acid),

12.2 2,4,6-trinitroaniline,

12.3 2,4,6-trinitrochlorobenzene.

13. A compound as claimed in Claim 6 wherein:-

5      $X^7$  is a group of formula-COQ in which Q is hydroxy, optionally substituted amino or has the formula -OR<sub>3</sub> in which R<sup>3</sup> is alkyl or metal;

$X^8$  is hydrogen or halogen;

10

$X^9$  and  $X^{10}$  are, each independently, hydrogen, halogen or nitro;

$X^{11}$  is hydrogen; and

15

$X^{12}$  is hydrogen, nitro, optionally substituted amino or halogen.

14. A compound as claimed in Claim 13 and as set forth  
20 below by name:-

14.1 2-chloro-5-nitrobenzoic acid

14.2 2,4-dichloro-3,5-dinitrobenzoic acid or its alkyl ester

14.3 4-chloro-3,5-dinitrobenzoic acid or its alkyl ester

25 14.4 2,5-dichlorobenzoic acid

14.5 2,4-dinitrobenzoic acid

14.6 3,5-dinitrobenzoic acid

14.7 3-nitro-4-chloroanisole

- 5     14.8   4-amino-3,5-dinitrobenzamide  
      14.9   4-chloro-3,5-dinitrobenzamide  
      14.10   2,4-dichloro-3,5-dinitrobenzamide

15.   A compound as claimed in Claim 6 wherein:

10

$X^7$  is optionally substituted amino; and

$R^8$  to  $R^{12}$  are, each independently, hydrogen, halogen or  
nitro.

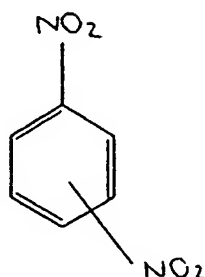
15

16.   A compound as claimed in Claim 15 and as set forth  
below by name:-

- 16.1   2,6-dinitroaniline  
20     16.2   2,4-dinitroaniline  
      16.3   3,5-dinitroaniline  
      16.4   2,4-dinitro-6-chloroaniline  
      16.5   2,6-dinitro-4-chloroaniline  
      16.6   2-chloro-4-nitroaniline  
25     16.7   2,4-dinitro-5-fluoroaniline

17.   A compound as claimed in any one of Claims 1 to 5  
wherein a ring hydrogen provides a labile moiety, the

5 compound having the general formula:



(VIII)

10

18. A compound as claimed in Claim 17 and as set forth by name below:-

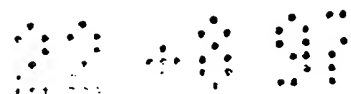
15 18.1 1,2-dinitrobenzene

18.2 1,3-dinitrobenzene

18.3 1,4-dinitrobenzene

19. A compound as claimed in any one of Claims 2 to 5,  
20 wherein at least one of  $X^1$  to  $X^6$  is selected from:-

|    |                        |   |
|----|------------------------|---|
| 25 | labile moiety/moieties | - 1 or 2 halo groups<br>and/or $NH_2$ or<br>substituted $NH_2$ and/or<br>COOH or substituted COOH<br>and/or alkyl and/or<br>$SO_3H/SO_3M$ |
|----|------------------------|---|



- 5 electrophilic moiety/moieties - 1 or 2 nitro groups  
and/or SO<sub>3</sub>H/SO<sub>3</sub>M and/or  
1 or 2 halo groups

20. A compound for use in the treatment or prevention of  
10 cancer, pre-cancer or disease caused by viral infection,  
which compound comprises an aromatic ring structure having  
at least one labile leaving moiety and at least one  
electrophilic moiety.

15 21. A compound for use in the treatment or prevention of  
cancer, pre-cancer or disease caused by viral infection,  
the compound being selected from the following classes of  
organic compounds:-

- 20 21.1 chlorodinitrobenzenesulphonic acid  
21.2 chlorobenzenesulphonic acid  
21.3 dichlorobenzenesulphonic acid  
21.4 aminodinitrobenzenesulphonic acid  
21.5 nitromethylbenzenesulphonic acid  
25 21.6 glutathionyldinitrobenzenesulphonic acid  
21.7 nitrochlorobenzenesulphonic acid  
21.8 dinitrobenzenesulphonic acid  
21.9 dinitrochlorobenzene  
21.10 dinitrofluorobenzene

- 5    21.11 dichlorodinitrobenzene
- 21.12 trinitrophenol e.g. picric acid
- 21.13 trinitroaniline
- 21.14 trinitrochlorobenzene
- 21.15 trinitrobenzenesulphonic acid
- 10   21.16 chloronitrobenzoic acid
- 21.17 chlorodinitrobenzoic acid
- 21.18 dichlorobenzoic acid
- 21.19 dichloronitrobenzoic acid
- 21.20 dichlorodinitrobenzoic acid
- 15   21.21 dinitrobenzoic acid
- 21.22 nitrochloroanisole
- 21.23 aminodinitrobenzamide
- 21.24 dinitroaniline
- 21.25 dinitrochloroaniline
- 20   21.26 chloronitroaniline
- 21.27 dinitrofluoroaniline

22. A compound for use in the treatment or prevention of cancer, pre-cancer or disease caused by viral infection,
- 25 the compound being a compound as set forth below by name:-

- 22.1 2,4,6-trinitrophenol
- 22.2 2,4-dichloro-3,5-dinitrobenzoic acid
- 22.3 4-chloro-3,5-dinitrobenzoic acid

5     23. A compound for use in the treatment or prevention of cancer or pre-cancer, the compound being a compound as set forth below by name:-

23.1 1,5-dichloro-2,3-dinitrobenzene

10    23.2 2-chloro-5-nitrobenzoic acid

23.3 4-chlorobenzenesulfonic acid

23.4 4-chloro-3,5-dinitrobenzene sulfonic acid

24. A compound for use in the treatment or prevention of  
15    disease caused by viral infection, the compound being a compound as set forth below by name:-

24.1 4-chloro-3,5-dinitrobenzamide

24.2 2,4-dichloro-3,5-dinitrobenzamide

20

25. A pharmaceutical composition, which composition comprises a compound according to any preceding claim and a pharmaceutically-acceptable diluent or carrier.

25    26. A composition as claimed in Claim 25, wherein the diluent or carrier is aqueous.

27. A composition as claimed in Claim 25 or Claim 26 which is in unit dosage form.



5

28. A composition as claimed in Claim 27 which is in the form of a tablet, capsule, powder, solution or suspension.

29. Use of a compound as claimed in any one of Claim 1 to  
10 24 for the preparation of a medicament for the prophylaxis or therapy of cancer, pre-cancer or viral infection.

30. Use as claimed in Claim 29 wherein the compound is at a concentration and dose which enables an arylating  
15 mechanism to be brought into play.

31. A method of treating disease caused by viral infection, which method comprises administering an effective amount of a compound as claimed in any one of  
20 Claims 1 to 24 or a composition as claimed in any one of Claims 25 to 28.

32. A method of treating cancer or pre-cancer to reduce or eliminate cancerous growth, which method comprises  
25 administering an effective amount of a compound as claimed in any one of Claims 1 to 24 or a composition as claimed in any one of Claims 25 to 28.

- 5     33. A chloro- or nitro-benzenesulfonic acid compound, a chloro- or nitro-benzoic acid compound or chloro- or nitro-benzamide compound for use as a pharmaceutical.



Application No: GB 9715492.6 Examiner: S J Pilling  
Claims searched: 7,8, (1-6,11,19-21,23,25-33 Date of search: 20 August 1997  
in part)

**Patents Act 1977**  
**Search Report under Section 17**

**Databases searched:**

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.O): A5B (BHA)

Int CI (Ed.6): A61K

Other: ONLINE: CAS ONLINE

**Documents considered to be relevant:**

| Category | Identity of document and relevant passage   | Relevant to claims                               |
|----------|---|--|
| X        | GB 1283331 (SMITH-KLINE & FRENCH) see page 1 lines 9 to 76 and the examples.                          | Claims 1 to 8; 11, 19 to 21, 23, 25 to 30 and 33 |
| X        | EP 0040420 A1 (THE DOW CHEMICAL CO) see page 1 line 5 to page 2 line and the examples.                | Claims 1 to 8; 11, 19 to 21, 23, 25 to 30 and 33 |
| X        | WO 91/15200 A2 (AYUKO) see page 7 line 12 to page 9 line 21 and claims 3, 6 and 7.                    | Claims 1 to 8; 11, 19 to 21, 23, 25 to 30 and 33 |
| X        | Merck Index, 1989, 11th edition, Merck and Co., see page 1409 with reference to compound Number 8901. | Claims 1 to 8; 11, 19 to 21, 23, 25 to 30 and 33 |

|   |  |
|---|--|
| X Document indicating lack of novelty or inventive step   | A Document indicating technological background and/or state of the art.  |
| Y Document indicating lack of inventive step if combined with one or more other documents of same category. | P Document published on or after the declared priority date but before the filing date of this invention.          |
| & Member of the same patent family  | E Patent document published on or after, but with priority date earlier than, the filing date of this application. |

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